The Evaluation of Autosomal SNP Assays

SNPs (single nucleotide polymorphisms) have the potential to play a useful role in human identification. Small PCR amplicon sizes associated with SNP typing technologies make SNPs attractive for typing degraded DNA or other low copy number situations. SNP markers can be useful in combination with STRs for resolving complex paternity issues (e.g., incest), identifying victims of mass disasters where insufficient family references are available, and possibly inferring population of origin.

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In certain circumstances, such as when DNA is degraded, SNPs (single nucleotide polymorphisms) can be useful as a supplementary tool, complementary to STR markers. To help demonstrate their usefulness, NIST researchers have studied SNP markers from a total of ten populations world-wide.

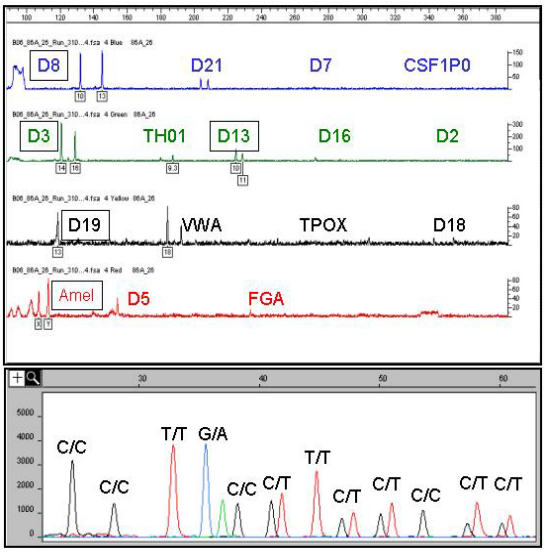
Initially a total of 189 samples from 3 different US populations (Caucasian, African American, and Hispanic) were typed for 70 autosomal bi-allelic (C/T) SNP markers. Each sample was typed with these genetic markers using 11 unique 6-plexes and a single 4-plex Polymerase Chain Reaction (PCR). Recently, an additional 375 samples from 7 global populations were typed with the 70 SNP markers.

SNP typing used for this work involved multiplex allele specific primer extension (ASPE) reactions. This assay includes an initial PCR amplification followed by primer extension and subsequent fragment separation and detection by capillary electrophoresis. Using this method, a SNP multiplex reaction was created containing 12 loci. These 12 loci were the minimum number of the 70 SNP loci needed to individually distinguish all 189 population samples. This information was based on observed heterozygosity and p-values obtained for Hardy-Weinberg equilibrium.

Over 1000 samples have been typed for both the 70 SNP markers and the 12-plex subset of markers. Further testing of the 12-plex assay with degraded samples revealed that these SNPs may provide useful information for challenging samples. Sensitivity studies with the SNP 12-plex displayed accurate typing results down to picomolar concentrations with pristine samples. In addition, testing the 12 loci with 1 ng of a 15-year-old bloodstain resulted in a full profile (Fig.1). This same bloodstain was also amplified with the Identifiler STR kit* and gave only partial typing results. Thus, the 12-plex autosomal SNP assay that we have designed can provide additional information beyond traditional STRs for degraded DNA samples.

However, some important considerations are the larger number of markers required to equal the discriminatory power compared to traditional STRs, their inability to resolve complex mixtures, issues related to databasing new loci, and the availability of a standard analysis platform. SNP markers will unlikely replace STR typing methods due to many of the factors listed above. However, results from this study show that SNP assays can complement STR typing methods to obtain increased sample information. These data benefit the forensic community when it is faced with selecting additional genetic markers and emerging genotyping technologies. Our future plans include the evaluation of potential challenges of sample mixture interpretation with SNPs compared to STRs.

Electropherograms of Identifiler and 12-plex SNP Assay Run on an Aged Blood Stain



Upper electropherogram: Identifiler genotyping result from a blood stain aged 15 years stored at room temperature. (Stored on #903 paper, Chelex extracted.) Only 4 STR loci gave full profiles.
Lower electropherogram: The same sample extract as above typed by the 12-plex SNP assay. All loci were successfully typed. The eleven different samples that gave partial profiles with Identifiler also gave full profiles typed with the 12-plex assay.

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